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BRIEF COMMUNICATION

A DISSECTION METHOD FOR DETERMINING THE GUT CONTENTS OF CALANOID COPEPODS

The examination and recording of gut contents has proved to be a useful aid in the study of the diets of zooplankton. The method cannot give a complete picture of the diet of a particular species as some food items are more delicate than others and are more readily broken during mastication and dissolved by digestive enzymes. However, many algae and animals in the diets of copepods remain sufficiently undamaged, or have adequate identifiable parts that are resistant to enzymatic breakdown, to allow a reasonably good assessment of diet from gut contents analysis [1,2,3,4]. It is preferable to base the analysis on the contents of the fore-gut (Fig. 2), in which much of the ingested material has been less affected by enzymatic action and is less compacted, than on the compacted and well digested bolus in the hind-gut, or the faecal pellets.

It is not a simple matter, however, to dissect out the entire gut contents of a copepod, largely because of the manipulative delicacy required. The contents of the foregut can be particularly difficult to extract in their entirety because of their diffuseness. Those who have used a dissection method may thus choose to remove only the hind gut bolus[5]. The methods most used for examining gut contents avoid direct dissection. In the squash technique, the gut contents are extracted from either live or preserved specimens by pressing down on a coverslip over the animal[1,2,6]. This method has the advantage of both releasing the material in the gut and dispersing it so that individual items may be identified and counted. Another method is to render the whole animal transparent by clearing it in lactic acid[7], or in cuparal or canada balsam after passing it through an alcohol series[8]. The drawback here is that the gut contents are not dispersed, and even when the gut boluses can be clearly seen the individual food items are mostly difficult to identify positively or to count. One way of overcoming such difficulties is to place the specimen under a cover slip and erode away most of the tissues with weak sodium hypochlorite[9]. The hypochlorite is then flushed away before the gut contents are oxidised, and it is usually possible to identify many of the individual food items by gently moving the cover slip, which partly redistributes the gut contents. The method has been used successfully in Australia to determine maximum gut food-particle sizes of the copepods *Calamoecia lucasi*, *Boeckella minuta* and *B. triarticulata* in Wallerawang Reservoir[4].

During a study of carnivory by three large omnivorous calanoid copepods *(Boeckella major, B. pseudochelae* and *Hemiboeckella searli)* from temporary ponds on the upper River Murray floodplain, we tried all the above methods of gut contents analysis. None of them proved entirely satisfactory, particularly for revealing the remains of animals in the gut contents. This appeared to be mainly due to the large size and thick bodies of the copepods, and because we had available only specimens preserved in 4% formalin and 70% alcohol. The squash method appeared reasonably satisfactory for small specimens, but in larger animals (and particularly those preserved in 4% formalin) the gut contents were often difficult to observe clearly amongst the mass of disrupted exoskeleton and muscle tissue. Clearing in lactic acid was only partly successful. The copepods did not clear very well, again apparently because of their large size. Whenever the food boluses could be seen clearly, animal remains (e.g. cuticle, setae) were difficult or impossible to recognise as they usually were crushed and compacted within the bolus. The hypochlorite erosion method was also not entirely successful. Even though the gut contents could be partly manipulated, the fact that the gut boluses were not fully dispersed made animal material difficult to see. As well, it was found that bubbles of oxygen produced during tissue erosion accumulated within the body and obscured the gut contents, and that care had to be taken to ensure that the gut contents themselves were not oxidised.

In order to overcome these difficulties we developed the following dissection method, which enables the entire gut contents of both small and large copepods to be removed. The contents of both fore- and hind-guts can be cleanly extracted without interference from most surrounding tissues, dispersed, and permanently mounted.

Needles for dissection are made from 2 cm lengths of 0.3 mm tungsten wire, which is rigid enough to allow some pressure to be applied during dissection, and may be sharpened to a fine point. For dissecting large copepods a sharp enough point can be produced with a fine diamond whetstone (e.g. "Ezelap"). For small copepods it is better to produce the desired point by erosion, either in molten $NaNO_3$, heated over a bunsen burner in a crucible[10] or by electrolysis in 10-20% KOH. For electrolysis, the wire is clamped to one terminal of a 6V alternating current electrical circuit (a microscope-illumination transformer is suitable) and dipped into the KOH[11,12]. In either case, the wire is moved in and out of the fluid, and the depth to which the wire is inserted governs whether the resulting point is short and stout or long and slender. The sharpened needle is then mounted in a holder. Satisfactory holders may be made from pin vices (small finger-operated drill holders available from model shops) that have been lengthened, if necessary, by the addition of a section of brass rod (Fig. 1,e). The tungsten needle is bent at a slight angle to the axis of the holder (Fig. 1,tn), to aid keeping the needle parallel to the slide surface during dissection. Jeweller's forceps, with finely sharpened points, are used for transferring copepods, or their parts.

Dissection can be done in water, but it is easier if a more viscous medium is used. Polyvinyl alcohol-lactophenol mountant (PVA)[13] is very suitable as it can be used to make permanent mounts of the gut contents. Lignin pink may be added to the PVA to stain chitin.

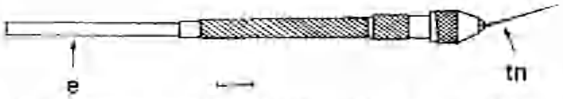


Fig. 1. A dissecting needle, consisting of a pin vice holding a finely pointed tungsten needle (tn). The commercially available pin vice has been extended by the addition of a section of brass rod (e). Scale bar = 1 cm.

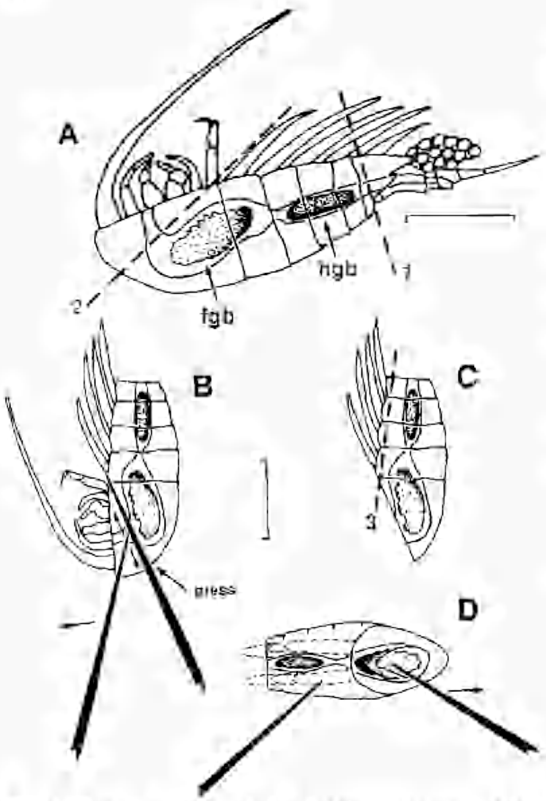


Fig. 2. Dissection of gut contents. Orientation of specimens is that for a right handed person. A. Lateral view of *Boeckella major* showing the fore gut (fgb) and hind gut (hgb) boluses. The first and second dissection cut-lines are shown by dashed lines 1 and 2, respectively. B. Orientation of the copepod and dissection technique for removing the antero-ventral portion of the prosome. The right needle is placed with its point between the maxilliped and first swimming legs and pressed firmly down and held against the slide. Back and forth movements of the left needle then sever the antero-ventral surface, which is pulled away to the left. C. The body ready for transfer to the second drop of PVA. The optional cut-line for removing the remaining ventral surface is shown by a dashed line. D. Extraction of the fore gut contents. The body is held with the left needle while the fore gut bolus is gently pulled out with the right needle. Scale bars = 1 mm.

Using the forceps, two drops of PVA are placed on a slide. The copepod is picked up with the forceps and placed in one drop, in which most of the dissection (i.e. removal of urosome, antero-ventral surface and mouthparts) is done. The body is then transferred to the second drop for the removal, teasing-out and mounting of the gut contents.

Dissection is done using a stereo dissecting microscope at a magnification of ca. 30-40×. The copepod is orientated with its ventral surface partially inclined to the left and away from the dissector, and, for a right-handed person, with its anterior end to the left (Fig. 2a). Firstly the urosome and terminal segment of the prosome are removed by cutting along dashed line 1 (Fig. 2a), and then the antero-ventral surface of the cephalosome plus mouthparts, by cutting along dashed line 2 (Fig. 2a). If desired, the swimming limbs (P1-P4) may also be removed (by cutting along dashed line 3, Fig. 2c). This is not absolutely necessary but may be useful if the ovaries are well developed. Swollen ovarioles make removal of the gut contents difficult, and removal of the swimming limbs and remaining ventral surface usually results in the concomitant removal of much of the ovary tissue.

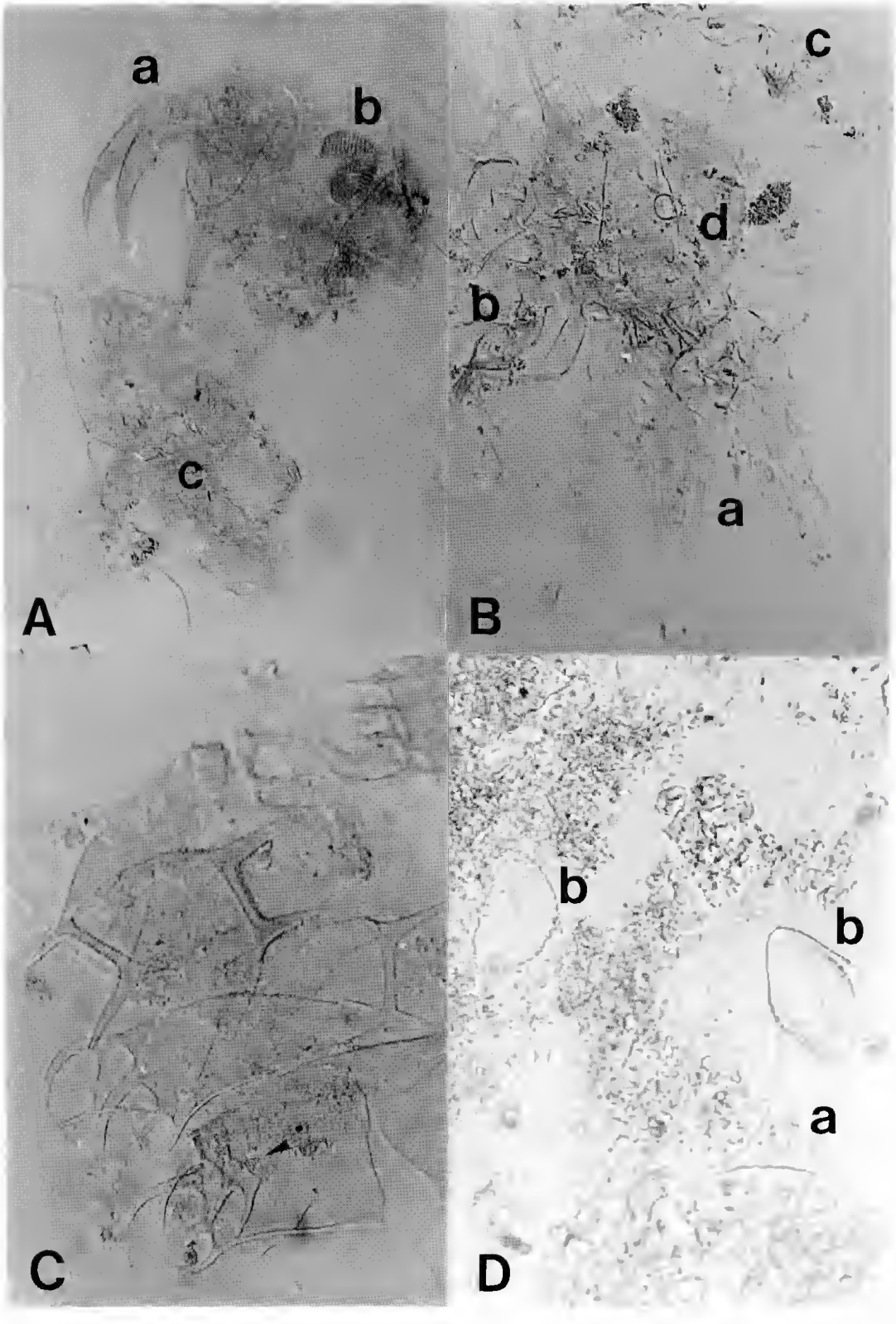
The first cut is made with the animal orientated as shown in Fig. 2a. The body is held with the left needle and the cut made with the right needle by pressing down firmly along line 1, using a forward and backward sawing action of the right needle if necessary. For the second cut, the animal is reorientated to the position shown in Fig. 2b. The animal is held with the left needle (near the base of the first antennae is a suitable point) and the right needle firmly pressed down over the body (Fig. 2b), with the point of the needle between the maxillipeds and first pair of swimming limbs. While the right needle is pressed firmly down against the slide, the antero-ventral surface and mouthparts are severed by back and forth cutting movements by the tip of the left needle (Fig. 2b). The procedure usually pushes the fore-gut bolus slightly dorso-posteriorly towards the rear of the fore gut, and very occasionally may result in the rear-gut bolus being extruded. If this happens, the rear-gut bolus can be retrieved with the forceps and transferred to the second drop of PVA. The body should now look as shown in Fig. 2c. If necessary, the swimming legs may now be removed by cutting along line 3 (Fig. 2c), pressing down on the body with the right needle.

Using the forceps, the body may now be transferred to the second drop of PVA, and held by the left needle with ventral side uppermost and anterior end facing right (Fig. 2d). The fore-gut contents are then carefully scraped out with the right needle (Fig. 2d), the body rotated 180°, and the rear-gut bolus removed in a similar manner. Finally, the body is removed with the forceps and discarded.

The food boluses may now be carefully teased apart with both needles and a small coverslip added. A 10 mm or smaller diameter coverslip is better than the standard 24 mm size, to reduce the area that has to be searched during microscope examination. The gut contents can be fully dispersed by the application of light pressure, and perhaps also small side-to-side movements, to the top of the cover slip with a needle or the forceps.

The gut contents of both small (e.g. *Boeckella symmetrica*, body length ca. 1-1.5 mm) and large (e.g. *B. major*, body length ca. 3-5 mm) freshwater calanoid copepods can be easily extracted using this dissection method. Because both the gut boluses can be extracted and teased apart we found that the

Fig. 3. Photomicrographs of animal remains and algae in dissected gut contents of *Boeckella major*. A. *Daphnia carinata*: a, post-abdomen; b, mandibles; c, cuticle and thoracic limbs. B. a, calanoid copepodite limbs; b, calanoid copepodite mandible; c, calanoid nauplius; d, *Testudinella patina*. C. *Keratella procurva*, trophus arrowed. D. Algae: a, *Staurastrum* sp; b, indet. diatom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method reveals animal remains in the gut contents better than the whole-animal squash and clearing techniques mentioned above. It is possible to pick out both very small animal remains (e.g. rotifer trophi, Fig. 3), and the diaphanous cuticular remnants and setae of cladocerans and copepods (Fig. 3). The visibility of cuticular fragments is enhanced by the lignin pink stain in the PVA, and also by the use of Nomarski interference optics. Algae, fungi, detritus and inorganic material in the guts are also clearly visible (Fig. 3). It is possible to make quantitative counts of the gut contents.

Animals preserved in 70% alcohol proved to be easier to dissect than those in 4% formalin. Alcohol preservation results in the dissolution of much of the muscle tissue and the softening of the exoskeleton. The body is thus easier to sever and to manipulate than when preserved in formalin, and there is less tissue "rubbish" in the final gut contents preparation.

The dissected limbs and other body parts remaining in the first drop of PVA can be put to good use. The mouthparts can be dissected off the remnant antero-ventral surface more readily than they can be from the whole animal. To do this, the apodemes at the bases of the mouthparts are anchored solidly against the slide with the left needle, while the mouthparts are easily dissected off with the right needle. Moreover, egg sacs removed with the urosome can be used for clutch-size determinations and measurements of egg size.

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